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Non-Contact Microrheology of Monolayers and Membranes
Roie Shlomovitz[1], Tom Boatwright[2], Michael Dennin[2], Alex J. Levine[1].
[1]University of California, Los Angeles, Los Angeles, CA, USA,
[2]University of California, Irvine, Irvine, CA, USA.
Understanding the mechanics/rheology of surface monolayers and lipid bilayers is of fundamental biological importance. One technique used to explored these questions is membrane microrheology, in which the observed thermal fluctuations of a tracer particle in the monolayer is used to extract the rheological data. This technique is challenging for at least two reasons. On the one hand, in fragile monolayer systems the presence of the tracer can locally perturb the monolayer. On the other hand, in sufficiently stiff monolayers/membranes it has proved problematic to embed the particle in it. In this talk, we develop a noncontact microrheological approach to avoid these issues by exploring the effect membrane rheology on the thermal fluctuations of a bead in the fluid near the monolayer.
Specifically, we develop the theory of the force response function of a spherical particle submerged below either a Langmuir monolayer or a lipid bilayer. We show that one can use the observed thermal fluctuations of that submerged particle to extract rheological properties of the essentially two-dimensional monolayer or membrane. We also present experimental results on application of this technique to surfactant monolayers and monolayers bound to an F-actin network on the aqueous side of the Langmuir monolayer.

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Ultrasound-Induced Currents in Planar Lipid Blayers: Origins and Potential Physiological Significance
Martin L. Prieto, Omer Oralkan, Butrus T. Khuri-Yakub,
Merritt C. Maduke.
Stanford University, Stanford, CA, USA.
Low-intensity focused ultrasound shows great promise for non-invasive, spatially resolved modulation of neural activity *in vivo*. To determine the mechanisms involved in ultrasonic modulation of neural activity and guide the development of this technology, we have been investigating the effects of ultrasound on protein-free planar lipid bilayers. Previously, we reported that ultrasound causes decaying current oscillations in planar bilayers at the onset and offset of the stimulus. These on and off responses are of opposite polarity but otherwise identical. Here, we report that if the rise time of the ultrasound pulse is prolonged, the on response is resolved into two distinct components: a sigmoidal component during the rise time and a damped oscillating component once the pulse reaches its final value. This result suggests that changes in ultrasound intensity during the rise time of the pulse may be important in determining the response to ultrasound *in vivo*, and is consistent with the observation that pulsed ultrasound is more effective than continuous ultrasound in modulating neural activity. To investigate further the origins of the on/off behavior, we used an optical interferometer to measure the velocity of the ultrasound-induced movement (acoustic streaming) in the solution surrounding the bilayer. We find that the time course of the ultrasound-induced current matches the time course of the streaming velocity, with a ratio of 162 pA/(mm/s). This acoustic streaming is probably due to the action of ultrasonic radiation force. To explore the potential physiological relevance of these effects, and to obtain further mechanistic insight, we are investigating the response of planar bilayers to ultrasound under current-clamp. In preliminary experiments, we find that ultrasound pulses with intensity comparable to those used *in vivo* produce voltage changes that would be sufficient to initiate an action potential.

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The Role of the Membrane Confinement for Cell Morphology and Surface Area Regulation
Margarita M. Staykova[1], Marino Arroyo[2], Mohammad Rahimi[2],
Howard A. Stone[1].
[1]Princeton University, Princeton, NJ, USA, [2]BarcelonaTech,
Barcelona, Spain.
Commonly, the membranes of mammalian and plant cells are not isolated, but rather they are confined to an actin cortex on the inner side and to an extracellular matrix, cell wall or a substrate on the outer side. The confinement restricts the modes of the membrane deformation and so influences the mechanisms for shape remodeling and surface area regulation. To study the mechanics of confined membranes we developed an in vitro system, which couples a lipid bilayer to the strain-controlled deformation of an elastic sheet (Staykova et al., PNAS 108:9084-9088, 2011). We demonstrate that upon contracting the elastic support, membrane protrusions grow out of the membrane plane, thus reducing its area; upon stretching, the bilayer absorbs the protrusions and expands its area without the lost of integrity. Most of our observations can be understood in terms of free energy minimization in a theoretical model we develop to account for stretch and curvature elasticity of each monolayer, the adhesion with the substrate, and the limited amount of area and volume available to the protrusions. However, some of the observed morphologies are dynamical in nature, and simulations suggest that the friction between the lower monolayer and the substrate plays a key role. Our experimental and theoretical results closely reproduce membrane processes found in, for example, shrinking neurons, and plant and muscle cells, and thus offer a mechanistic approach towards understanding the area regulation in cells.

Platform: Cardiac Electrophysiology

171-Plat
Novel Roles of Fibroblast Growth Factor Homologous Factors in Heart
Jessica Amenta, Geoffrey S. Pitt.
Duke University, Durham, NC, USA.
Genetic studies of inherited arrhythmias have highlighted essential physiologic roles of modulator proteins that affect ion channel pore-forming subunits. One such class of modulators is the fibroblast growth factor (FGF) homologous factors (FHFs; FGF11-14), that, despite their homology with FGFs, are incapable of functioning as growth factors, are intracellular modulators of Na^+ channels and are associated with neurodegenerative diseases. Unexpectedly, recent work in the Pitt lab has shown that mutations in FHFs affect voltage-gated calcium channels also, indicating that the FHFs can modulate multiple ion channels. The Pitt lab has recently characterized the role of the prominent heart FHF, FGF13, in modulating cardiac Na^+ channel function and trafficking, and further, $FGF13^{X/-}$ mice show a widened QRS and aberrant atrial conduction, therefore we hypothesized that FHFs regulate other ion channels in heart as well, particularly the L-type calcium channel (LTCC, $Ca_v1.2$).
In these studies, we use whole-cell patch clamp to show that in both a heterologous expression system and mouse ventricular cardiomyocytes, FGF13 affects $Ca_v1.2$ current density and kinetics. Immunostaining for the LTCC when FGF13 is knocked down results in aberrant localization of the pore-forming α-subunit, α_{1C}, and changes in T-tubule ultrastructure. Immunocytochemical analysis of adult mouse cardiomyocytes shows that FGF13 has a broad cellular distribution. Interestingly, a portion of the population colocalizes with Ankyrin B, a protein responsible for trafficking multiple ion channels in the heart.
These data of FHF's novel function in heart begin to define FHF's role in excitation-contraction coupling and cardiac arrhythmogenesis. These cellular mechanisms will lay the groundwork for in vivo electrophysiology studies on $FGF13^{X/-}$ mice.

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Illuminating Trafficking of KCNQ1/KCNE1 Channels in Heart
Ademuyiwa Aromolaran[1], William R. Kobertz[2], Henry M. Colecraft[1].
[1]Columbia University, New York, NY, USA, [2]University of Massachusetts
Medical School, Worcester, MA, USA.
In human heart, pore-forming KCNQ1 subunits associate with auxiliary KCNE1 subunits to generate the slowly activating, delayed rectifier potassium current, I_{Ks}. Decreases in I_{Ks}, due to either congenital mutations in KCNQ1/KCNE1 subunits or the adverse neuro-hormonal milieu accompanying heart failure, delay cardiac repolarization, leading to long QT syndrome (LQTS). LQTS predisposes to lethal ventricular arrhythmias and sudden cardiac death. How KCNQ1/KCNE1 channel trafficking in the heart proceeds and is regulated is unknown. However, understanding this process is critical both for fundamental mechanistic insights into LQTS, and for rational development of potential new therapies for this condition. We sought to develop optical tools that permit direct visualization of KCNQ1/KCNE1 subunit assembly and trafficking in heart cells. KCNQ1 and KCNE1 were tagged intra-cellularly with YFP and CFP, respectively, and extra-cellularly with a 13-residue high affinity α-bungarotoxin (BTX) binding site (BBS). The tagged proteins were viable as determined by functional electrophysiological assays in Chinese hamster ovary (CHO) cells– BBS-KCNQ1-YFP expressed alone gave rise to rapidly-activating outward currents, and co-expression with KCNE1-CFP resulted in the slowly activating kinetic signature of I_{Ks}. Cell surface BBS-tagged channel subunits were selectively detected with quantum dot. Optical pulse chase assays in human embryonic kidney (HEK 293) cells revealed that surface KCNQ1 channels undergo rapid endocytosis and recycling, and this process is largely unaffected by KCNE1. Tagged KCNQ1/KCNE1 subunits expressed in guinea pig heart cells using adenovirus were targeted to the surface sarcolemma, t-tubules and intercalated disks, similar to endogenous channels.